



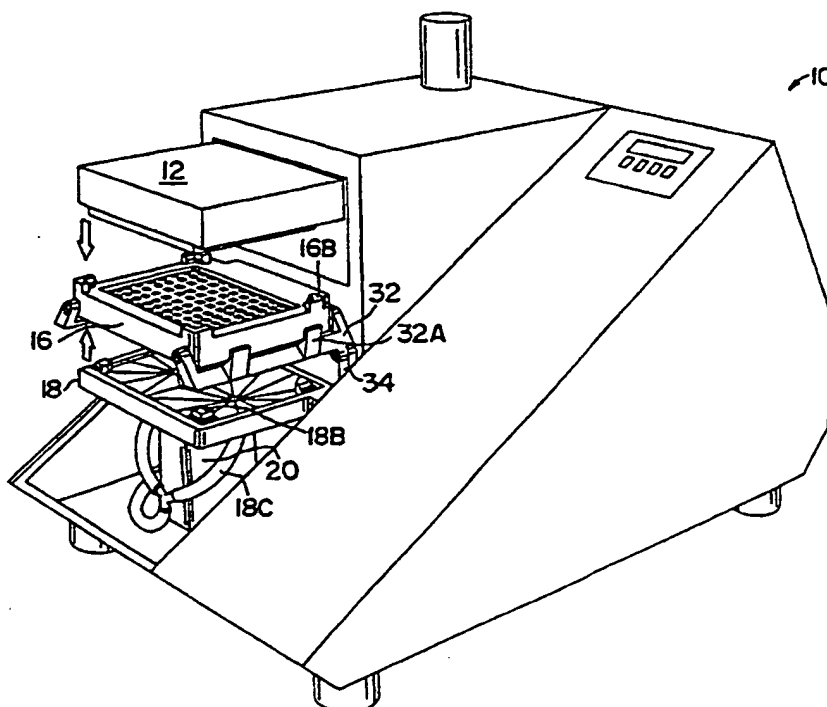
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : B01L 3/00, C12M 1/12, G01N 35/10		A1	(11) International Publication Number: WO 97/00136
			(43) International Publication Date: 3 January 1997 (03.01.97)
(21) International Application Number: PCT/US96/10264 (22) International Filing Date: 13 June 1996 (13.06.96) (30) Priority Data: 08/491,417 16 June 1995 (16.06.95) US (71) Applicant (for all designated States except US): GLAXO WELLCOME INC. [US/US]; Five Moore Drive, Research Triangle Park, NC 27709 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): BARGER, Lee, A. [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Re- search Triangle Park, NC 27709 (US). HARWARD, Brisco, L. [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). HOEPPNER, Douglas, K. [US/US]; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US). (74) Agents: LEVY, David, J. et al.; Glaxo Wellcome Inc., Five Moore Drive, Research Triangle Park, NC 27709 (US).		(81) Designated States: AL, AM, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TR, TT, UA, UG, US, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published With international search report.	

(54) Title: APPARATUS FOR AUTOMATED BIOLOGICAL CELL HARVESTING

(57) Abstract

An automated biological cell harvesting apparatus (10) comprising a fluid dispensing head (12) adapted to move to a lowered position and dispense fluid from a plurality of spray ports, a fixed upper tray (16) positioned beneath the fluid dispensing head (12) for supporting a biological filter plate (FP) of the type defining a plurality of fluid permeable wells therein, a vertically movable lower tray (18) adapted to move from a lowermost position to a raised position in engagement with the upper tray (16). Controller means is provided for dispensing fluid from the fluid dispensing head (12) into the wells of the filter plate (FP), and raising the lower tray (18) into its raised position in engagement with the upper tray (16) and then creating a vacuum between the lower tray (18) and the upper tray (16) to draw the fluid from the filter plate (FP) supported on the upper tray (16) into the lower tray (18) for collection in a microplate with a corresponding number of wells or, in the absence of a microplate, for removal of the fluid from the lower tray (18) to a remote common waste collection vessel.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 96/10264

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9111245	08-08-91	FR-A- 2657543	02-08-91
		AT-T- 110291	15-09-94
		DE-D- 69103621	29-09-94
		DE-T- 69103621	23-03-95
		EP-A- 0513139	19-11-92
		JP-T- 5504405	08-07-93
		US-A- 5306420	26-04-94
WO-A-9202303	20-02-92	AT-T- 131746	15-01-96
		DE-D- 69115674	01-02-96
		DE-T- 69115674	01-08-96
		EP-A- 0495066	22-07-92
		JP-T- 5502105	15-04-93
		US-A- 5273718	28-12-93
US-A-5380437	10-01-95	NONE	
US-A-4879431	07-11-89	JP-A- 3043075	25-02-91
WO-A-8300819	17-03-83	AT-A- 375841	10-09-84
		EP-A- 0086813	31-08-83
		JP-B- 3002264	14-01-91
		JP-T- 58501594	22-09-83
		US-A- 4559664	24-12-85
WO-A-9116979	14-11-91	EP-A- 0482165	29-04-92
		US-A- 5325609	05-07-94
US-A-4493896	15-01-85	NONE	
GB-A-2246081	22-01-92	US-A- 5141719	25-08-92
		CA-A,C 2043633	19-01-92
		DE-A- 4123874	23-01-92
		FR-A- 2664825	24-01-92
		IT-B- 1250493	08-04-95
		JP-A- 4227032	17-08-92



5

Description

APPARATUS FOR AUTOMATED BIOLOGICAL CELL HARVESTING

Technical Field

10

The present invention relates to an apparatus for biological cell harvesting, and more particularly to an automated apparatus for harvesting biological cells from fluid carried within biological filter plates.

Background Art

15

Laboratory scientists are familiar with the multi-step, semi-manual methods for biological cell harvesting from biological filter plates and the tedious and laborious effort involved in so doing. Recognizing the need for an automated biological cell harvesting apparatus to facilitate this procedure, applicants have developed the novel apparatus described hereinafter.

20

Very significantly, applicants discovered that an existing microplate washer, Model No. SLT 96PW available from Tecan USA, Inc. of Research Triangle Park, North Carolina, could be modified to achieve an entirely unintended function as an automated cell harvesting apparatus.

25

As known to those skilled in the art, the SLT 96PW microplate washer is a programmable microplate washer which utilizes an internal microprocessor control to wash/aspirate 96 well microplates. The MILLIPORE brand 96 well microplate is a well known brand of microplate, although other pharmaceutical support companies also distribute 96 well microplates.

5 In its original form, the SLT 96PW microplate washer apparatus comprises a fluid pump connected to a fluid dispensing head, a vacuum switch to initiate aspiration, a fixed tray for receiving a microplate to be washed, and a microcontroller for controlling the fluid dispensing and aspirating sequence achieved by 96 pairs of dispense/ aspirate tubes provided in the dispensing
10 head.

 The microcontroller of the SLT 96PW microplate washer provides flexibility to the apparatus by allowing for programmable variations in the soak and aspirate times as well as in the volume and speed of fluid dispensing. The wash process in the SLT 96PW includes the following programmable
15 parameters: the amount of fluid to dispense; the soak time; the aspirate time; and the number of times the cycle should be repeated for a microplate. As an option, the SLT 96PW includes a RS232 serial interface port that allows the apparatus to communicate synchronously with a personal computer or robot. In this mode, commands can be issued to the apparatus that specify the wash
20 program to run, and the SLT 96PW can send response signals to these commands with a variety of codes indicating the status of the apparatus.

 The fluid dispensing head of the SLT 96PW rides on a vertical track and has 96 pairs of stainless steel tubes depending downwardly from the bottom surface thereof. Each pair of stainless steel tubes includes a relatively shorter
25 tube to dispense fluid and a relatively longer tube to aspirate the well in which the pair of tubes is positioned. The vertical track and a precision electric stepper motor allow the fluid dispensing head to be optimally vertically

5 placed therein. Also, the apparatus does not have any capability to collect the fluid from the individual wells of a filter plate in distinct containers which is desirable in many laboratory studies.

The stainless steel fluid dispensing tubes direct the flow of liquid directly down into the wells of a filter plate in such a manner that the fluid streams have
10 a tendency to stir up biological cells that may have settled at the bottom of the wells. Also, in order for the original SLT 96PW to aspirate or remove liquid from the wells of a filter plate, the relatively long aspiration tubes must be immersed in the fluid. This dipping of the aspiration tubes into the fluid in the wells creates an opportunity for cross-contamination and, even more significantly, since there
15 is no mechanism to separate the cells from the liquid, the cells themselves can be removed along with the liquid.

Finally, in some studies it is beneficial to collect the fluid from each of the filter plate wells in corresponding distinct containers, and the SLT 96PW with its common aspiration manifold has no means of collecting fluid from each well in
20 a distinct container. Thus, these and other limitations which would be well known to one skilled in the art of cell harvesting would normally not lead one to attempt to use the SLT 96PW microplate washer as an apparatus for biological cell harvesting, a tedious procedure presently requiring the use of several devices and incorporating a number of manual steps.

25 Therefore, applicants' apparatus for automated biological cell harvesting is believed to fill a long-felt need for an automated apparatus for biological cell harvesting which obviates the need for the multiple step, semi-manual

5 positioned for introducing/ removing microplates and dispensing and removing fluid from the microplates. The 96 pairs of stainless steel tubes depending from the fluid dispensing head correspond to each of the 96 wells in sample microplates. Within each pair of stainless steel tubes, as noted hereinabove, one tube is used exclusively to dispense fluid from a common manifold, and the
10 other tube is used exclusively to remove the fluid from the well by suction (aspiration). Once the fluid is removed from the 96 wells of the microplate, it is collected as waste in a remote container.

The SLT 96PW uses a double-diaphragm electric pump driven by a stepper motor with an optical shaft encoder for delivery of fluid. In the dispense
15 mode, the pump can deliver between 25 and 300 microliters (ml) of liquid in 25 microliter (ml) increments. In the wash mode, the fluid pump can be programmed to deliver between 50 and 3000 microliters (ml) of liquid in 50 microliter (ml) increments, and the rate at which the fluid is dispensed can also be programmed into the apparatus.

20 The SLT 96PW has a manual, horizontally slidable tray for positioning microplates beneath the fluid dispensing head and a conventional LCD display to facilitate manual operation of the apparatus.

As would be appreciated by one skilled in the art of biological cell harvesting, the SLT 96PW as provided by the manufacturer was not intended
25 for use as a cell harvester and cannot do so due to certain inherent features. For example, the fluid dispensing head has a tendency to remove biological cells intended for harvesting when fluid is removed from the wells of a filter plate

5 procedure presently utilized in the art. The simple-to-use and highly reliable automated biological cell harvesting apparatus described and claimed hereinafter meets these and other long-felt needs in the art for such an apparatus.

Disclosure of the Invention

10

In accordance with the present invention, there is provided an apparatus for automated biological cell harvesting which comprises a vertically movable fluid dispensing head adapted to move from a raised position to a lowered dispensing position and including a plurality of spray nozzles depending from
15 the bottom surface of the head. A fixed upper tray is positioned beneath the fluid dispensing head and adapted to support a biological filter plate thereon having a plurality of fluid permeable wells therein corresponding to the plurality of dispensing head spray nozzles, and the upper tray being fluid permeable to allow fluid from the biological filter plate to flow therethrough. A vertically
20 movable lower tray is positioned beneath the upper tray and adapted to move from a lowered position beneath the upper tray to a raised position in engagement with the upper tray.

First actuator means is provided to move the dispensing head from the raised position to the lowered position, and second actuator means is provided
25 to move the lower tray from the lowered position to the raised position. A pump is provided to pump fluid through the fluid dispensing head when the dispensing head is in the lowered position, and vacuum means is provided to

5 create a vacuum between the upper and lower trays when the lower tray is moved to its raised position. This vacuum draws fluid from the wells of a biological filter plate supported on the upper tray into the lower tray so as to isolate biological cells within the wells of the filter plate. Finally, controller means is provided for selectively activating the first and second actuator means,
10 the pump means and the vacuum means according to a predetermined time sequence.

It is therefore an object of the present invention to provided an apparatus for automated biological cell harvesting to replace the multi-step, semi-manual cell harvesting procedure presently known in the art.

15 It is another object of the present invention to provide an apparatus for automated biological cell harvesting which is easy to use and provides highly reliable and reproducible cell harvesting results.

Some of the objects of the invention having been stated hereinabove, other objects will become evident as the description proceeds, when taken in
20 connection with the accompanying drawings as best described hereinbelow.

Brief Description of the Drawings

Figure 1 is a perspective view of the apparatus for automated biological cell harvesting according to the present invention;

25 Figure 2 is a bottom perspective view of the dispensing head of the apparatus shown in Figure 1;

5 Figure 3 is a perspective view of the upper and lower trays of the apparatus shown in Figure 1 with the bottom tray in its lowered position;

 Figure 3A is a view similar to Figure 3 but illustrating a second embodiment of the upper tray thereof;

 Figure 4 is a perspective view of the upper and lower trays of the
10 apparatus shown in Figure 1 with the lower tray in its raised position;

 Figure 5 is a perspective view similar to Figure 3 but showing a biological filter plate positioned on the upper tray;

 Figure 6 is a perspective view similar to Figure 4 but showing a biological filter plate positioned on the upper tray;

15 Figure 7 is a front elevation view of the upper and lower trays shown in Figure 5;

 Figure 8 is a front elevation view of the upper and lower trays shown in Figure 6;

 Figure 9 is a simplified schematic of the electronic and pneumatic control
20 mechanisms of the apparatus shown in Figure 1; and

 Figure 10 is an electrical schematic of the accessory board shown in Figure 9.

Best Mode for Carrying Out the Invention

25

Modifications To SLT 96PW Washer

Applicants discovered that an SLT 96PW microplate washer available from Tecan USA, Inc. of Research Triangle Park, North Carolina could be

5 modified so as to perform the biological cell harvesting function in an automated fashion. This discovery is quite significant since heretofore the cell harvesting procedure is a multi-step, semi-manual procedure which leaves much to be desired. Although the detailed description relates to modification of the SLT 96PW apparatus, applicants contemplate that the novel modifications could be
10 made to other similar apparatus or an original automated cell harvesting apparatus could be manufactured incorporating the novel features described herein.

Referring now to Figure 1-10 of the drawings, the apparatus for automated biological cell harvesting is shown in Figure 1 and generally
15 designated 10. Apparatus 10 comprises a vertically movable fluid dispensing head 12 which is vertically actuated by electric motor 14. A fixed upper tray 16 for supporting a biological filter plate thereon is positioned beneath fluid dispensing head 12, and a vertically movable lower tray 18 is provided below upper tray 16 and vertically actuated by pneumatic cylinder 20, most suitably a
20 PHD, Inc. brand, Part No. COA1C3/4 x 11/2 - U1 pneumatic cylinder. A liquid pump 22 is fluidly connected to dispensing head 12 and provides fluid thereto upon actuation. The motherboard controller 24 acts to actuate dispensing head 12, liquid pump 22 and vacuum valve 26. Applicants have used the signal that actuates vacuum valve 26 to control 4-way solenoid valve 30, most suitably a
25 HUMPHREY PRODUCTS brand Part No. H030-4E1 solenoid valve, via the accessory board circuit 28 (see Figure 9). 4-way solenoid valve 30 server to actuate pneumatic cylinder 20.

5 The new componentry and circuitry of applicants' apparatus can best be appreciated with reference to Figure 9 wherein it can be seen that all of the circuitry is conventional to the SLT 96PW washer except for accessory board circuit 28, 4-way solenoid valve 30 and pneumatic cylinder 20 which are all actuated by virtue of the electrical connection of accessory board circuit 28 (see
10 schematic in Figure 10) to the line leading from motherboard controller 24 to vacuum valve 26. Although a specific circuit for accessory board 28 is shown in Figure 10, applicants believe other circuits could be utilized as a matter of design choice and thus do not wish to limit the instant invention thereto.

 An apparatus suitable for biological cell harvesting must be capable of
15 dispensing a known fluid volume and removing the fluid from the 96 individual filter plate wells with minimal disturbance to the biological cells. The apparatus must also be capable of collecting the liquid from each filter plate well in a corresponding distinct container. Although applicants describe a 96 well filter plate and 96 nozzle dispensing head herein, applicants do not wish to be
20 limited to this specific number of filter plate wells or dispensing head nozzles but use it for purposes of illustration only.

 In order to prevent agitation of biological cells in the bottom of the filter plate wells, fluid dispensing head 12 must be modified so that the flow from the common manifold is not directed straight down but is angled so that it sprays
25 against the sides of the filter plate wells. Although this can be accomplished in a number of ways, applicants utilize a machined dispensing head plate 12A (shown in Figure 2) that provides 96 ducts, nozzles or ports (hereinafter referred

5 to as "nozzles") which each direct fluid flow from the nozzle outwardly through 2
diverging apertures and each aperture defines a spray angle of about 45° to the
surface of head plate **12A**.

In order to separate the liquid from the biological cells in 96 well filter
plate **FP**, the prior art utilizes commercially available, hand-operated units
10 wherein vacuum is applied to the bottom of the filter plates to draw the liquid
through the filter in the bottom of each well so as to leave only the biological
cells in the 96 wells. Applicants' apparatus **10** allows for placing filter plate **FP**
into upper tray **16** which has a bottom surface defining 96 apertures **16A**
therein in vertical registration with the bottom of the 96 wells of filter plate **FP**. A
15 bottom surface is most suitably provided to upper tray **16** as shown in Figure 3
in order to support the bottom of filter plate **FP** during the vacuum removal of the
fluid from the wells thereof as described hereinbelow. An alternative
embodiment is shown in Figure 3A wherein upper tray **16** defines a
substantially open bottom when the additional support of filter plate **FP** may not
20 be necessary. Upper tray **16** includes raised corners **16B** to facilitate
alignment of filter plates **FP** within upper plate **16** during use of apparatus **10**.

As a matter of procedural choice, a conventional solid bottom 96 well
microplate can be placed on lower tray **18** beneath filter plate **FP** to catch the
liquid in 96 corresponding and distinct containers as it is pulled from the
25 individual wells of filter plate **FP** during the vacuum step of apparatus **10**. If
collection of the fluid in distinct containers is not desired, a microplate is not
positioned on lower tray **18** and the fluid removed from filter plate **FP** can flow

5 through the two apertures **18A** in the bottom of lower tray **18** into a common waste collection vessel (not shown).

For the collection of liquid in distinct containers, the bottom microplate should be positioned on lower tray **18** over self-aligning posts **18B** to assure proper positioning of the fluid collection microplate. The bottom of lower tray **18**
10 is most suitably tapered toward apertures **18A** so that when collection of fluid in the individual containers of a microplate is not desired, the fluid will flow to the low points where vacuum lines **18C** (see Figure 7) are attached. Also, upper tray **16** defines a collar around the bottom surface thereof which acts to form a tight union with elastomeric seal **18D** provided around the top surface of lower
15 tray **18** when upper tray **16** and lower tray **18** are engaged and vacuum applied during the fluid removal step of apparatus **100**.

A pneumatic cylinder **20** is used to raise lower tray **18**, although an electric motor or other suitable mechanism can be utilized. Lower tray **18** will be used only when aspirating filter plate **FP** in upper tray **16** in order to harvest
20 the cells therein. The electrical signal for raising lower tray **18** is acquired from the SLT 96PW vacuum valve **26**. Accessory board circuit **28** acts to trigger the rise of bottom tray **18** when the vacuum valve initiates the vacuum. Accessory board circuit **28** also acts to lower tray **18** after the vacuum is released by solenoid vacuum valve **26**. Accessory board **28** is designed to provide a short
25 delay between releasing the vacuum and lowering tray **18** to facilitate equalization of the pressure between upper tray **16** and lower tray **18**.

- 5 Equalization of pressure is aided through a small hole in the wall of the upper tray **16** (not shown).

Detailed Description of Modifications To SLT 96PW Washer

- 10 Applicants provide a new nozzle manifold and nozzle plate or dispensing head plate **16A** to replace the original dispensing head and stainless steel tubes of the SLT 96PW apparatus. Dispensing head plate or nozzle plate **12A** has 96 nozzles **N** (see Figure 2) wherein each nozzle **N** has two holes drilled on a 45° angle to direct the fluid flow to the sides of the filter plate wells. The
- 15 two holes are supplied with fluid through a blind hole drilled through the upper side of dispensing head plate **12A**, and the blind holes transport the liquid from the manifold block to all 192 ports of 96 nozzles **N**. Nozzles **N** are most suitably configured with tapered edges to minimize the amount of fluid collecting on dispensing head plate **12A**, but could also be fabricated using small bore
- 20 stainless steel "Y" shaped nozzles. Nozzles **N** allow the ports to be lowered just below the lips of the filter plate wells in order to reduce splashing within the wells.

- The original sliding tray of the SLT 96PW has been replaced by fixed upper tray **16** (see Figures 3, 3A, 4-8). Upper tray **16** has four corners or posts
- 25 **16B** that act to align filter plate **FP** with dispensing head plate **16A**. Upper tray **16** also has a seal **16C** to form an air tight union between filter plate **FP** and upper plate **16**. As noted hereinbefore, upper tray **16** defines a collar along the bottom surface thereof to engage seal **18D** around the top perimeter surface of

5 lower tray **18** (see Figures 3, 3A and 5). Also, the collar of upper tray **16** acts to encase a microplate carried by lower tray **18** when the lower tray is raised into the vacuum forming position with upper tray **16**. As previously noted, posts **18B** are provided at the corners of lower tray **18** to facilitate aligning a conventional 96 well microplate therein with filter plate **FP** supported
10 thereabove by upper tray **16**. The bottom surface of lower tray **18** is tapered towards two apertures **18A** to which vacuum lines **18C** are attached to reduce the amount of fluid retained on the surface of lower tray **18** when a collection microplate is not utilized in the cell harvesting procedure. Applicants have also designed a custom accessory board circuit **28** (see Figures 9 and 10) to control
15 the raising and lowering of bottom tray **18**. Accessory board circuit **28** monitors the line on the SLT 96PW motherboard controller **24** that fires vacuum solenoid valve **26** so that when the line goes high, accessory board circuit **28** activates 4-way solenoid valve **30** to allow compressed gas to raise pneumatic cylinder **20**. When the line on motherboard controller **24** goes low, accessory
20 board circuit **28** delays for approximately 2 seconds and then switches 4-way solenoid valve **30** again to redirect the air flow through the 4-way solenoid valve so that lower tray **18** is vertically lowered. The 2 second delay allows time for enough air to pass through the bleed hole located on the front of upper tray **16** (not shown) to relieve the vacuum within the chamber formed by upper
25 tray **16**, lower tray **18**, and filter plate **FP** (or optionally, upper plate **16**, lower plate **18**, filter plate **FP** and a microplate carried on lower plate **16**).

5 Biological filter plates **FP** are frequently slightly bowed they do not
inherently seal well without some assistance. Applicants have addressed this
issue by providing a mechanism for clamping filter plate **FP** to upper tray **16**
with a clamp mechanism as best seen in Figures 3, 3A-4 and 5-8. Clamping
mechanism **30** consists of a pivotably mounted upper linkage **32** provided at
10 each end of upper tray **16** which is caused to pivot upwardly by pivot arm **34**
pivotably mounted at one end to lower tray **18** and at the other end to upper
linkage **32**. Thus, when lower tray **18** is caused to move vertically upwardly,
upper linkage **32** at each end of upper tray **16** pivots upwardly so as to cause
fingers **32A** to pivot into position against opposing ends of filter plate **FP**.
15 Fingers **32A** at each end of filter plate **FP** act to rotate into position against filter
plate **FP** (between the two layers of filter plate **FP**) and to lightly press down on
the bottom of filter plate **FP** to flatten the plate and form a satisfactory seal of
filter plate **FP** against seal **16C** of upper tray **16**.

Pneumatic cylinder **20** that acts to vertically motivate lower tray **18** is
20 energized from a pressurized gas source (not shown) through a hole in the
housing of apparatus **100**. The gas most suitably passes through a pressure
regulator and flow regulator (not shown) to limit the speed and force with which
lower tray **18** is moved. The original vacuum line of the SLT 96PW has been
re-routed by applicants from the back of dispensing head **12** to vacuum lines
25 **18C** of lower tray **18**.

5

Method of Use

Apparatus 10 for automated biological cell harvesting is used in somewhat the same manner as the original SLT 96PW microplate washer from which it is derived. The electrical, fluid and vacuum lines are attached to apparatus 10 in much the same way as the original microplate washer apparatus. A pressurized gas source has been added, however, to drive pneumatic cylinder 20, and it is attached to the rear of apparatus 10 with a connector (not shown). Motherboard controller 24 has not been modified (other than tapped for an electrical connection to accessory board 28) and acts to control apparatus 10 with the same commands as it controlled the SLT 96PW microplate washer.

The primary difference occurs whenever a signal is sent to vacuum valve 26. When the original SLT 96PW apparatus is asked to "aspirate" or wash a microplate, the head is lowered to a predetermined height associated with a particular microplate type and vacuum to the head is actuated. As the stainless steel tubes lower into the solution within the wells, the liquid is drawn out by the aspiration tubes through the vacuum manifold and vacuum valve and out of the apparatus where it is collected in a trap on the vacuum line.

In contrast, when vacuum valve 26 of automated cell harvesting apparatus 10 is activated, dispensing head 12 is lowered as before. Simultaneously with the opening of vacuum valve 26, applicants' new accessory board circuit 28 acts to raise lower tray 16. As lower tray 16 rises, clamping mechanism 30 acts to pivot fingers 32A into position against filter

5 plate **FP**. Once fingers **32A** and lower tray **18** are in place, a chamber is created where a vacuum can form and air is removed from the chamber by repositioned vacuum lines **18C** (see Figure 7). As pressure is lowered in the chamber, the pressure differential forces the fluid in the 96 well filter plate above the chamber through the well filters, and the removed liquid can then proceed to
10 exit the system and be collected as waste or (if a microplate is carried on lower tray **18**) it can be collected in a 96 well microplate.

After a preset amount of time is passed, accessory board circuit **28** terminates the vacuum. Lower tray **18** is held in position for approximately 2 seconds to allow the chamber's pressure to equalize with the ambient presser.
15 The equalization occurs as air continues to pass through both the filters of filter plate **FP** and the small hole (not shown) on the front of the chamber defined in upper tray **16**. The hole assures that the pressure will equalize even if filter plate **FP** filters are clogged or otherwise air impermeable. If the delay is not present, the low pressure over the relatively large area of the chamber will
20 prevent small pneumatic cylinder **20** from pulling lower tray **18** down.

Thus, applicants' apparatus for automated cell harvesting maintains many of the features of the SLT 96PW microplate washer from which it is derived, but the modifications thereto surprisingly allow for extracting solids from liquids in filter plates. The liquid extracted from the wells of the filter plates
25 can be collected either in mass or in the 96 distinct containers of a separate microplate. In this fashion novel apparatus **100** provides automated cell

- 5 harvesting whereas previously the procedure was a tedious, multi-step semi-manual procedure.

It will be understood that various details of the invention may be changed without departing from the scope of the invention. Furthermore, the foregoing description is for the purpose of illustration only, and not for the purpose of
10 limitation--the invention being defined by the claims.

5

CLAIMS

What is claimed is:

1. An automated apparatus for harvesting materials from fluid carried by a filter plate, comprising:

- 10 (a) a movable fluid dispensing head with a top and a bottom surface adapted to move from a first position to a second position and having a plurality of dispensing means on the bottom surface thereof;
- 15 (b) a fixed upper tray positioned beneath and spaced apart from said fluid dispensing head and adapted to support a filter plate thereon comprising a plurality of fluid permeable wells therein corresponding to said dispensing head plurality of dispensing means, and said upper tray being fluid permeable to allow fluid from said filter plate to flow therethrough;
- 20 (c) a movable lower tray positioned beneath said upper tray and adapted to move from a first spaced-apart and lowered position beneath said upper tray to a second raised position in engagement with said upper tray;
- 25 (d) first actuator means adapted to move said fluid dispensing head from said first position to said second position and back to said first position, and pump means to pump fluid through said fluid dispensing head when said fluid dispensing head is moved to its second position by said first actuator means;

5 (e) second actuator means adapted to move said lower tray from said first lowered position to said second raised position and back to said first lowered position, and vacuum means to create a vacuum between said upper and lower trays when said lower tray is moved to its second raised position by said second actuator means so as to draw fluid from the plurality of wells of a filter plate supported on said upper tray into said lower tray to isolate material within said wells of said filter plate; and

10

(f) controller means for selectively activating said first and second actuator means and said pump means and said vacuum means according to a predetermined time sequence.

15

2. An apparatus according to claim 1 including clamping means operatively connected to said upper tray for securing a filter plate thereto when said lower plate is raised into engagement with said upper tray.

3. An apparatus according to claim 1 wherein said

20 dispensing means is formed to provide one or more spray streams.

4. An apparatus according to claim 3 wherein said one or more spray streams comprises a plurality of spray streams directed outwardly from each other.

5. An apparatus according to claim 1 wherein said upper tray

25 comprises a bottom support surface defining a plurality of apertures therein corresponding to the plurality of wells in a filter plate supported by said upper tray.

5 6. An apparatus according to claim 1 wherein said upper tray defines an opening in the bottom thereof extending beneath the plurality of wells in a filter plate supported on said upper tray.

 7. An apparatus according to claim 1 wherein said lower tray comprises at least one aperture in the bottom thereof for applying a vacuum to
10 said upper tray when raised to said second raised position.

 8. An apparatus according to claim 7 wherein said lower tray is adapted to optionally carry a microplate thereon which defines a plurality of wells therein corresponding to said plurality of fluid permeable wells in said filter plate so that fluid drawn from said filter plate wells will be received in said
15 corresponding plurality of microplate wells.

 9. An apparatus according to claim 1 wherein said first actuator means is an electric motor and said pump means is an electric liquid pump.

 10. An apparatus according to claim 1 wherein said second actuator means is a pneumatic cylinder and said vacuum means is a vacuum pump in
20 operative connection with a vacuum valve.

 11. An apparatus according to claim 1 wherein said controller means is a microprocessor circuit for automatically activating said first actuator means and pump means, and said second actuator and said vacuum means according to a predetermined timing sequence.

25 12. An apparatus according to claim 11 wherein said controller means is adapted to provide for selective manual control of the automated apparatus

- 5 for selectively changing the timing sequence for cell harvesting or for other use applications such as microplate washing.

13. An automated apparatus for harvesting biological cells from fluid carried by a biological filter plate, comprising:

- 10 (a) a vertically movable fluid dispensing head with a top and a bottom surface and adapted to move from a first raised position to a second lowered dispensing position and having a plurality of spray nozzles on the bottom surface thereof wherein each spray nozzle is formed to provide a pair of spray streams directed outwardly from each other and each of said pair of spray streams defines a 45° angle with the bottom surface of said fluid dispensing head;
- 15 (b) a fixed upper tray positioned beneath and spaced apart from said fluid dispensing head and adapted to support a biological filter plate thereon of the type defining a plurality of fluid permeable wells therein corresponding to said dispensing head plurality of spray nozzles, and said upper tray being fluid permeable to allow fluid from said biological filter plate to flow therethrough;
- 20 (c) a vertically movable lower tray positioned beneath said upper tray and adapted to move from a first spaced-apart and lowered position beneath said upper tray to a second raised position in engagement with said upper tray;
- 25

- 5 (d) first actuator means adapted to move said fluid dispensing head from said first raised position to said second lowered position and back to said first raised position, and pump means to pump fluid through said fluid dispensing head when said fluid dispensing head is moved to its second lowered position by said first actuator means;
- 10 (e) second actuator means adapted to move said lower tray from said first lowered position to said second raised position and back to said first lowered position, and vacuum means to create a vacuum between said upper and lower trays when said lower tray is moved to its second raised position by said second actuator means so as to draw fluid from the plurality of wells of a biological filter plate supported on said upper tray into said lower tray to isolate biological cells within said wells of said biological filter plate;
- 15 (f) clamping means operatively connected to said upper tray for securing a biological filter plate thereto when said lower plate is raised into engagement with said upper tray; and
- 20 (g) a microprocessor for selectively activating said first and second actuator means and said pump means and vacuum means according to a predetermined time sequence.
- 25

14. An apparatus according to claim 13 wherein said plurality of spray nozzles comprise 96 spray nozzles.

5 15. An apparatus according to claim 13 wherein said upper tray comprises a bottom support surface defining a plurality of apertures therein corresponding to the plurality of wells in a biological filter plate supported by said upper tray.

10 16. An apparatus according to claim 13 wherein said upper tray defines an opening in the bottom thereof extending beneath the plurality of wells in a biological filter plate supported on said upper tray.

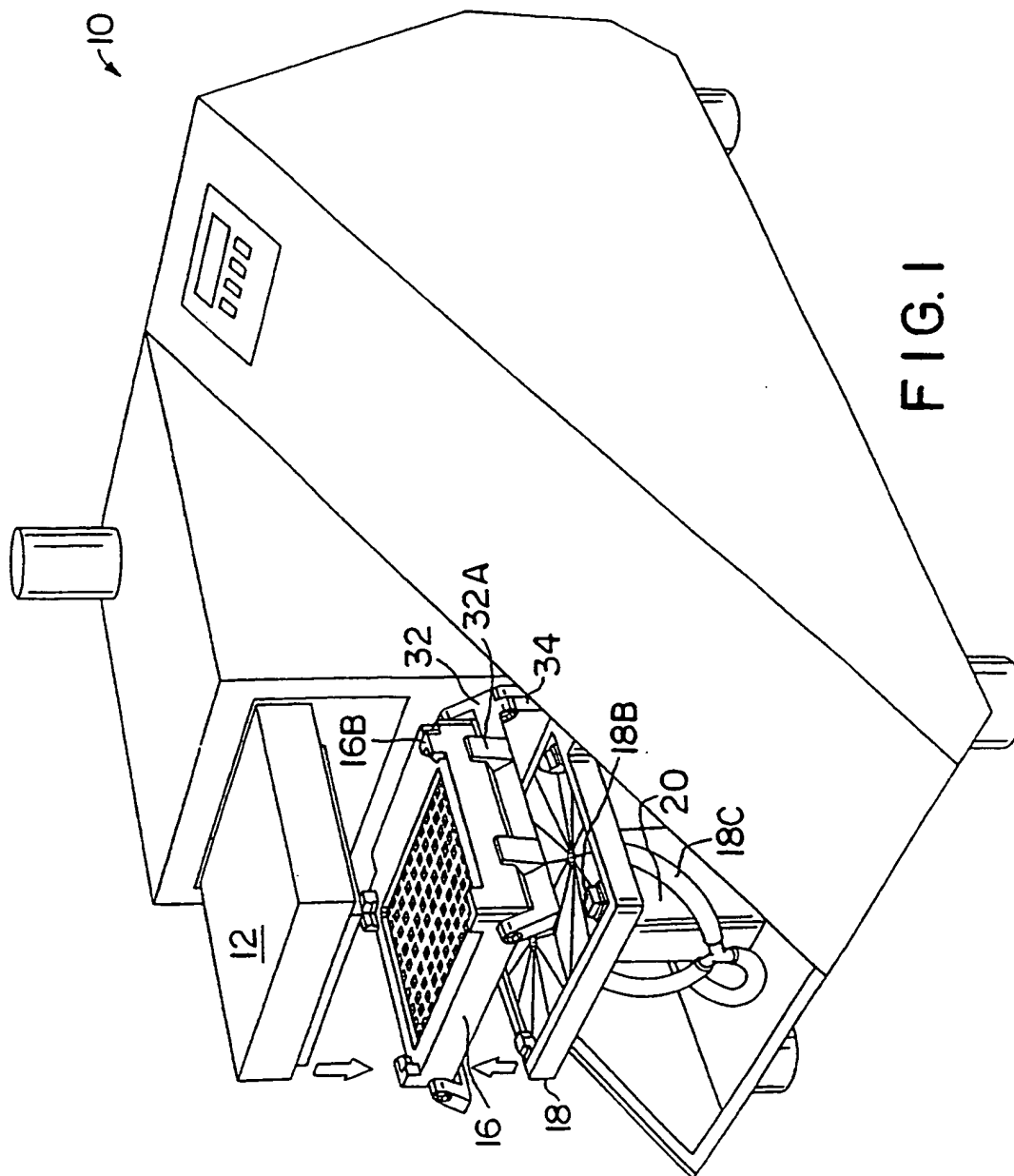
 17. An apparatus according to claim 13 wherein said lower tray defines at least one aperture in the bottom thereof for applying a vacuum to said upper tray when raised to said second raised position.

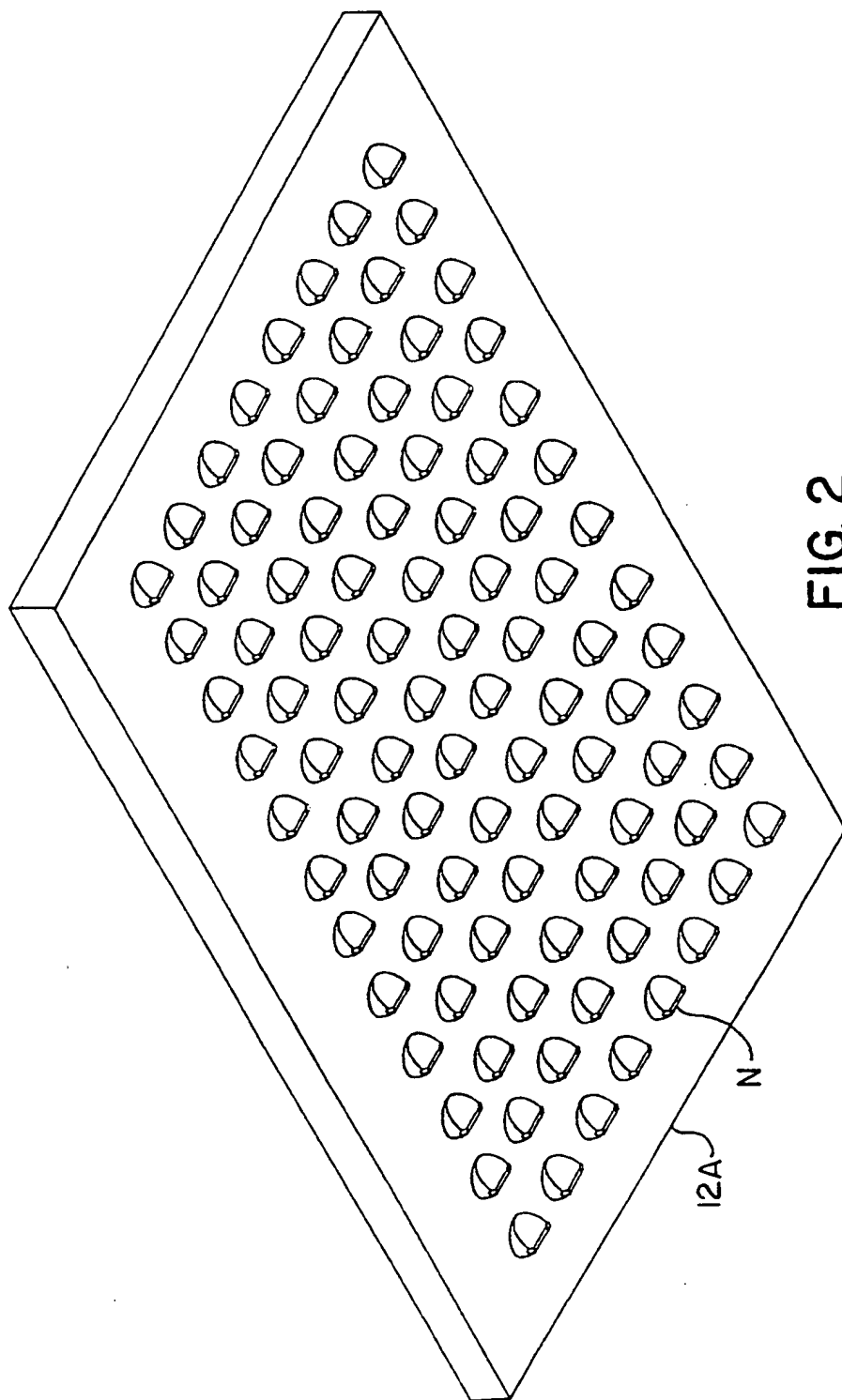
15 18. An apparatus according to claim 17 wherein said lower tray is adapted to optionally carry a microplate thereon which defines a plurality of wells therein corresponding to said plurality of fluid permeable wells in said filter plate so that fluid drawn from said filter plate wells will be received in said corresponding plurality of microplate wells.

20 19. An apparatus according to claim 13 wherein said first actuator means is an electric motor and said pump means is an electric liquid pump.

 20. An apparatus according to claim 13 wherein said second actuator means is a pneumatic cylinder and said vacuum means is a vacuum pump in operative connection with a vacuum valve.

- 5 21. An apparatus according to claim 13 wherein said microprocessor is adapted to provide for selective manual control of the automated apparatus for selectively changing the timing sequence for all harvesting or for other use applications such as microplate washing.





3/8

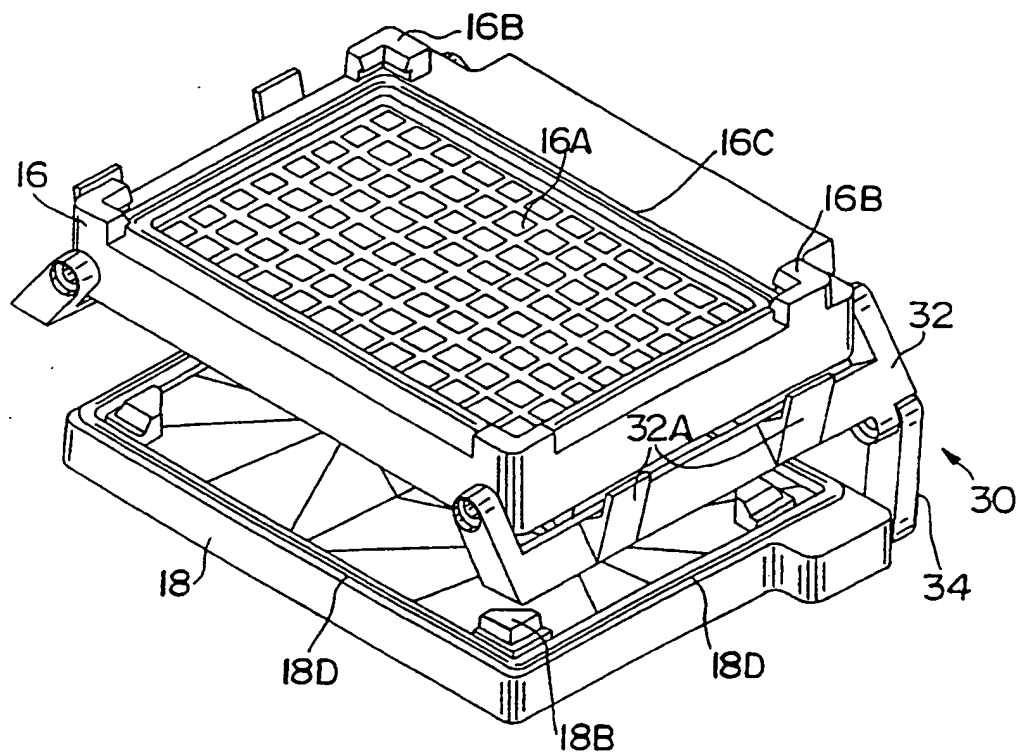


FIG. 3

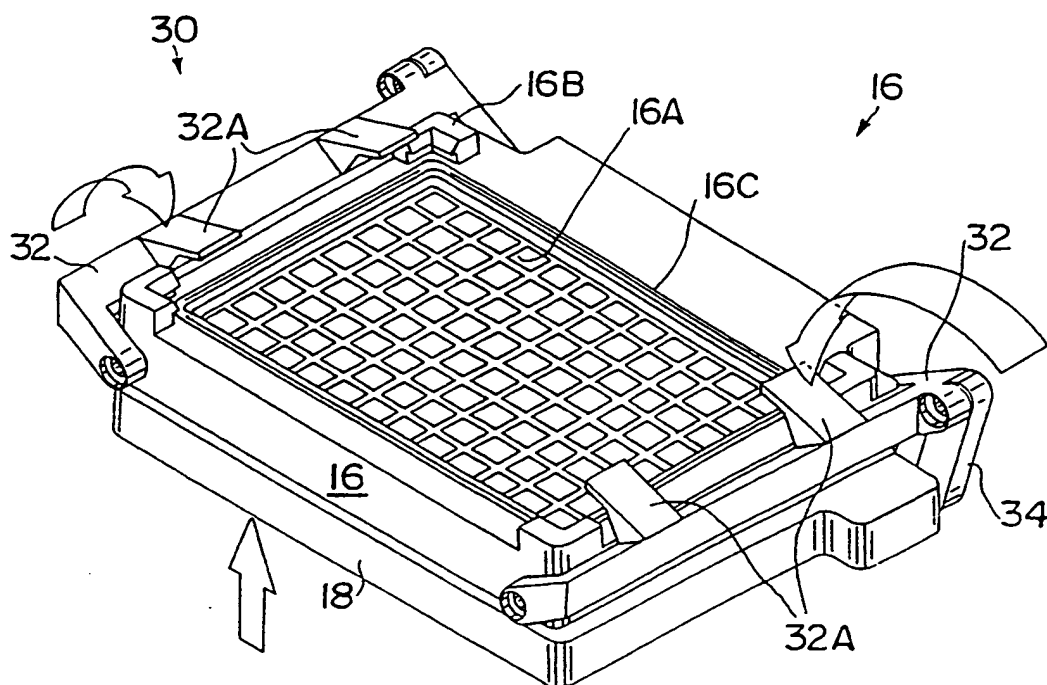


FIG. 4

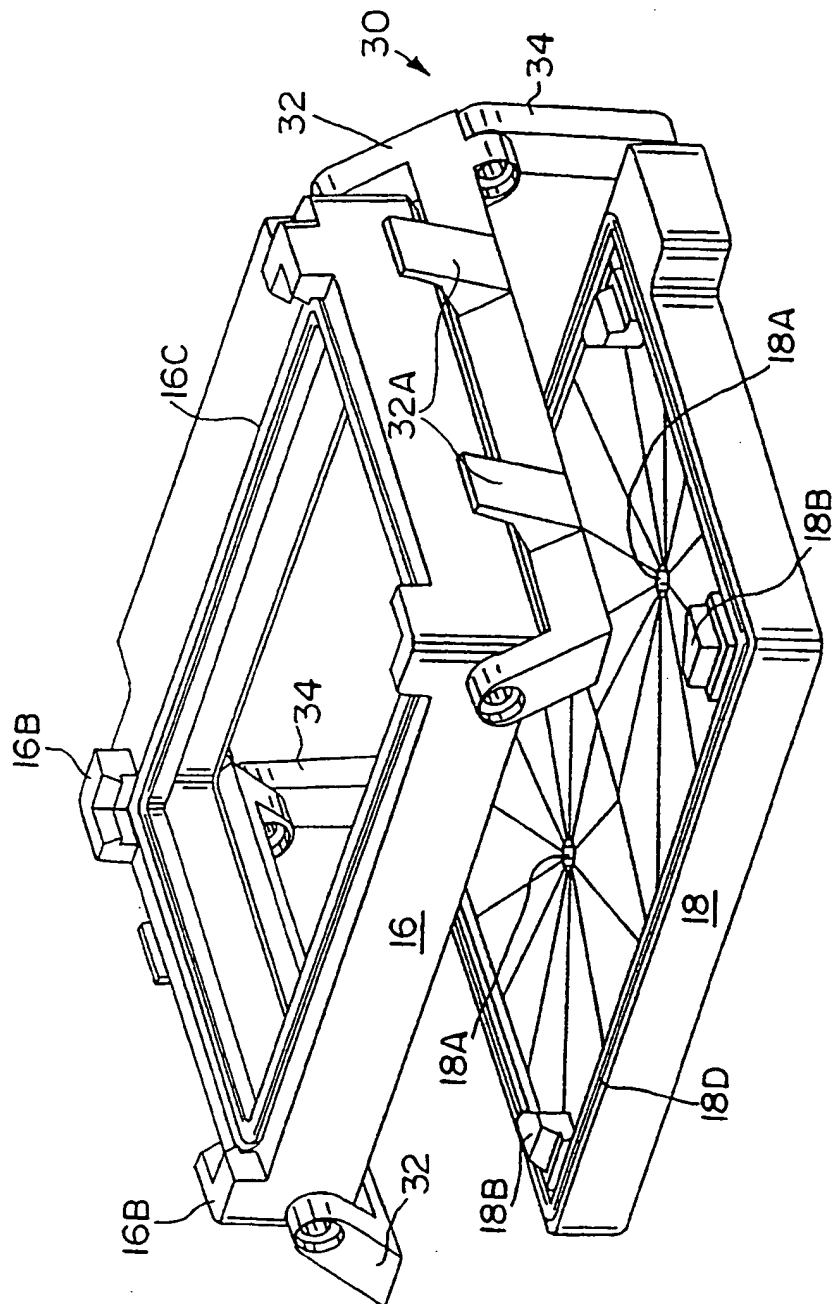
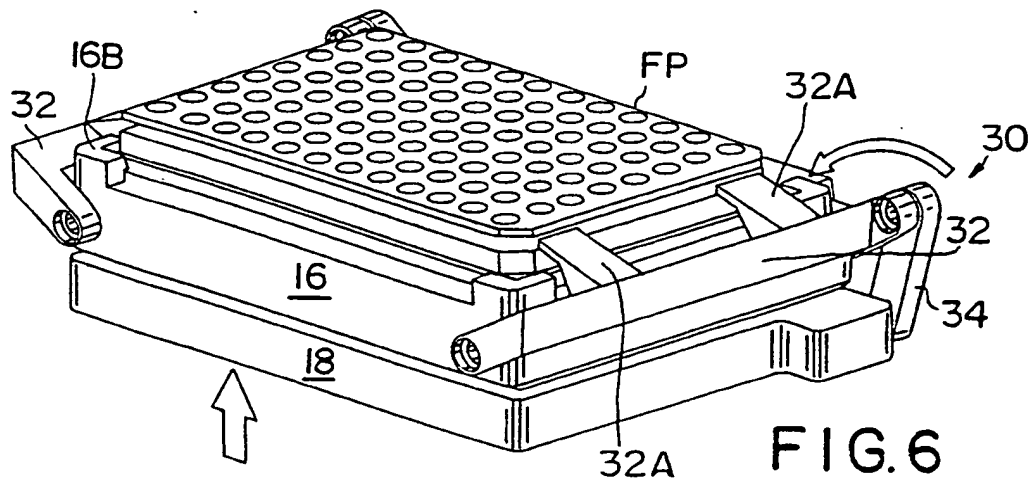
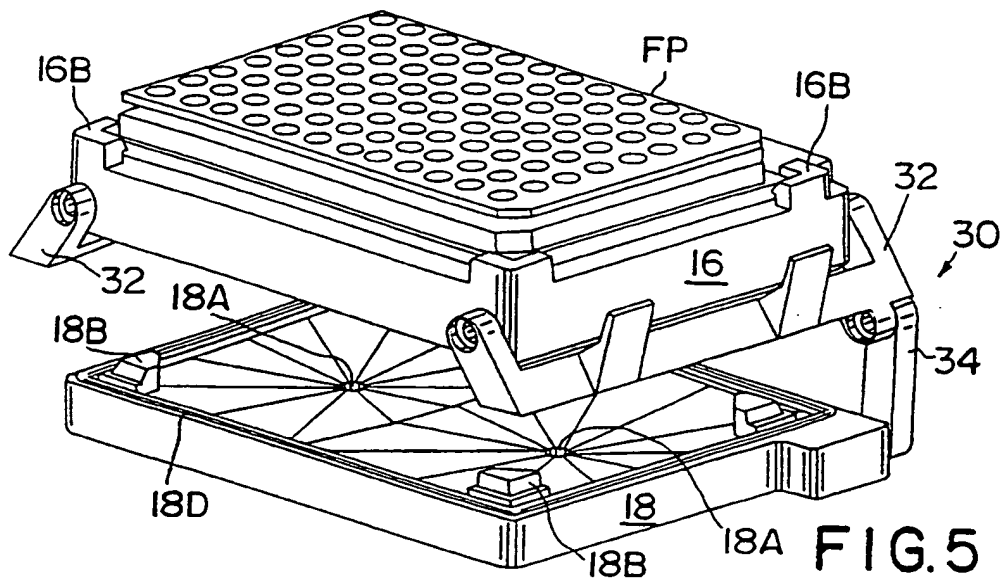
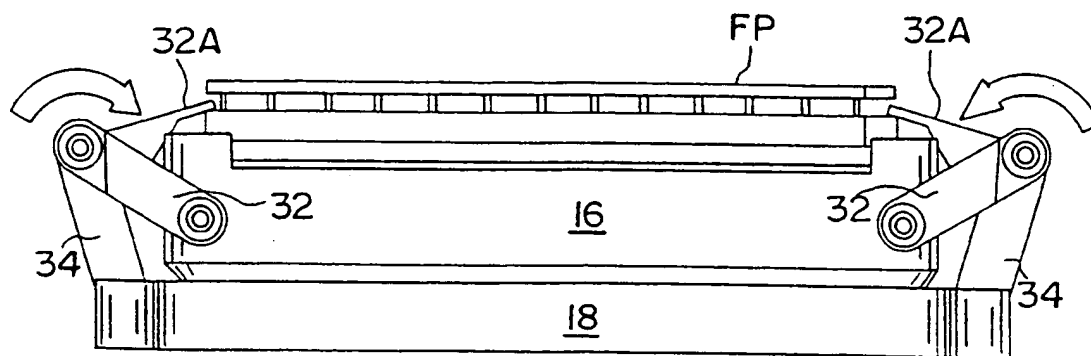
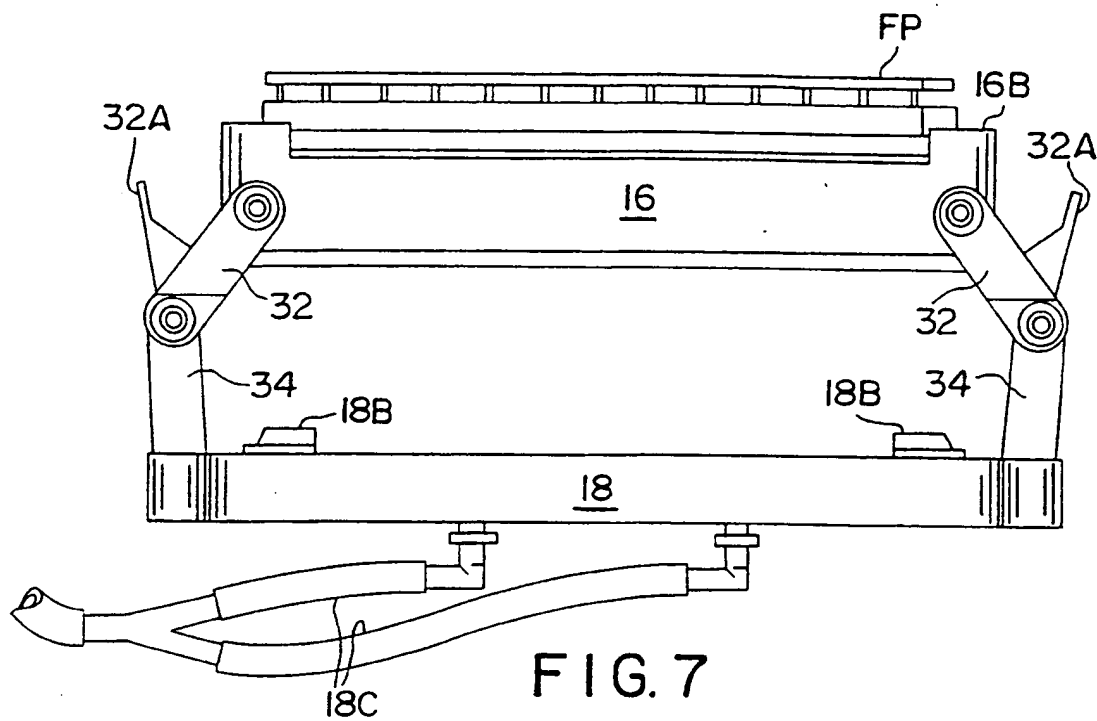


FIG. 3A





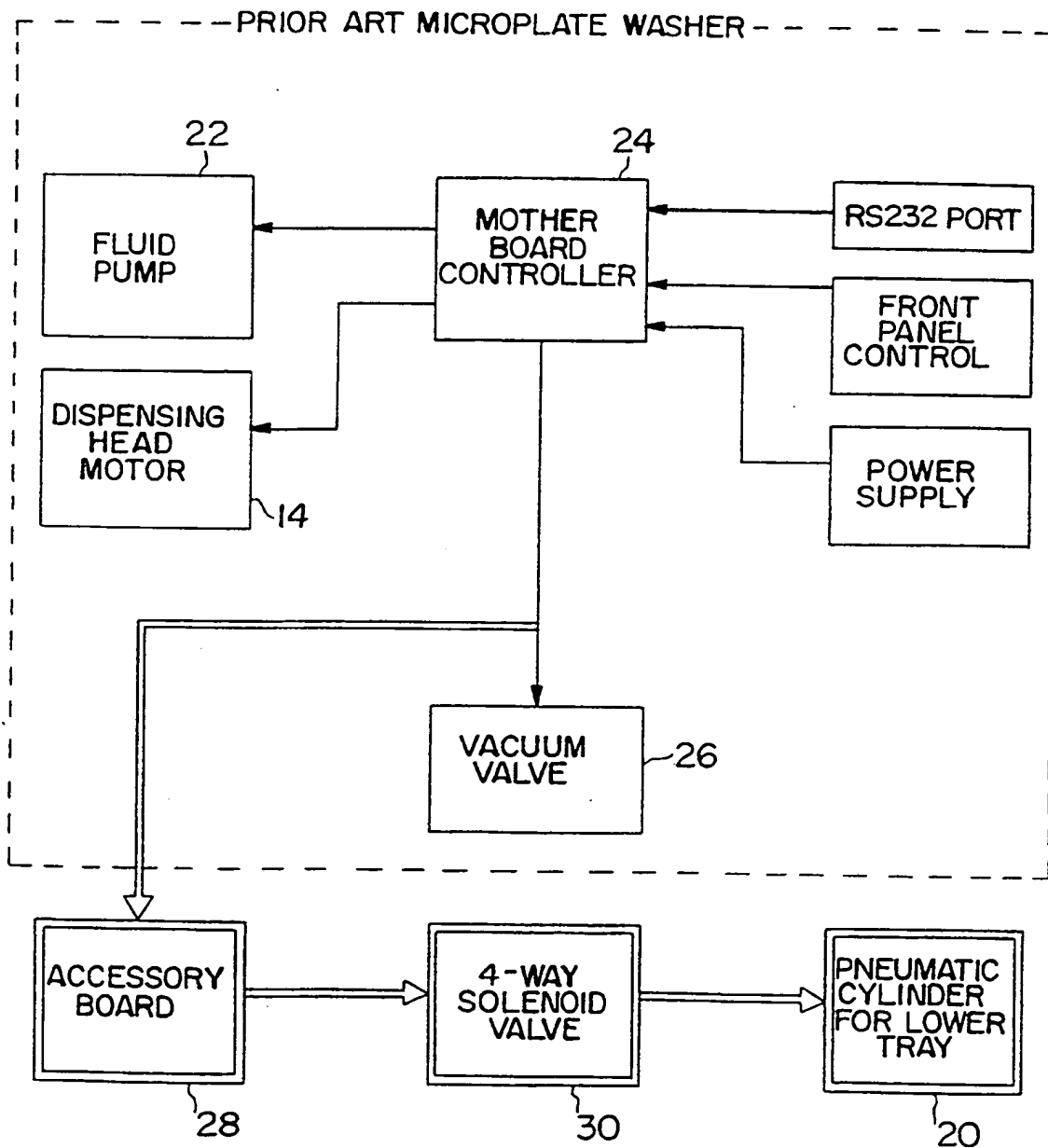


FIG. 9

8/8

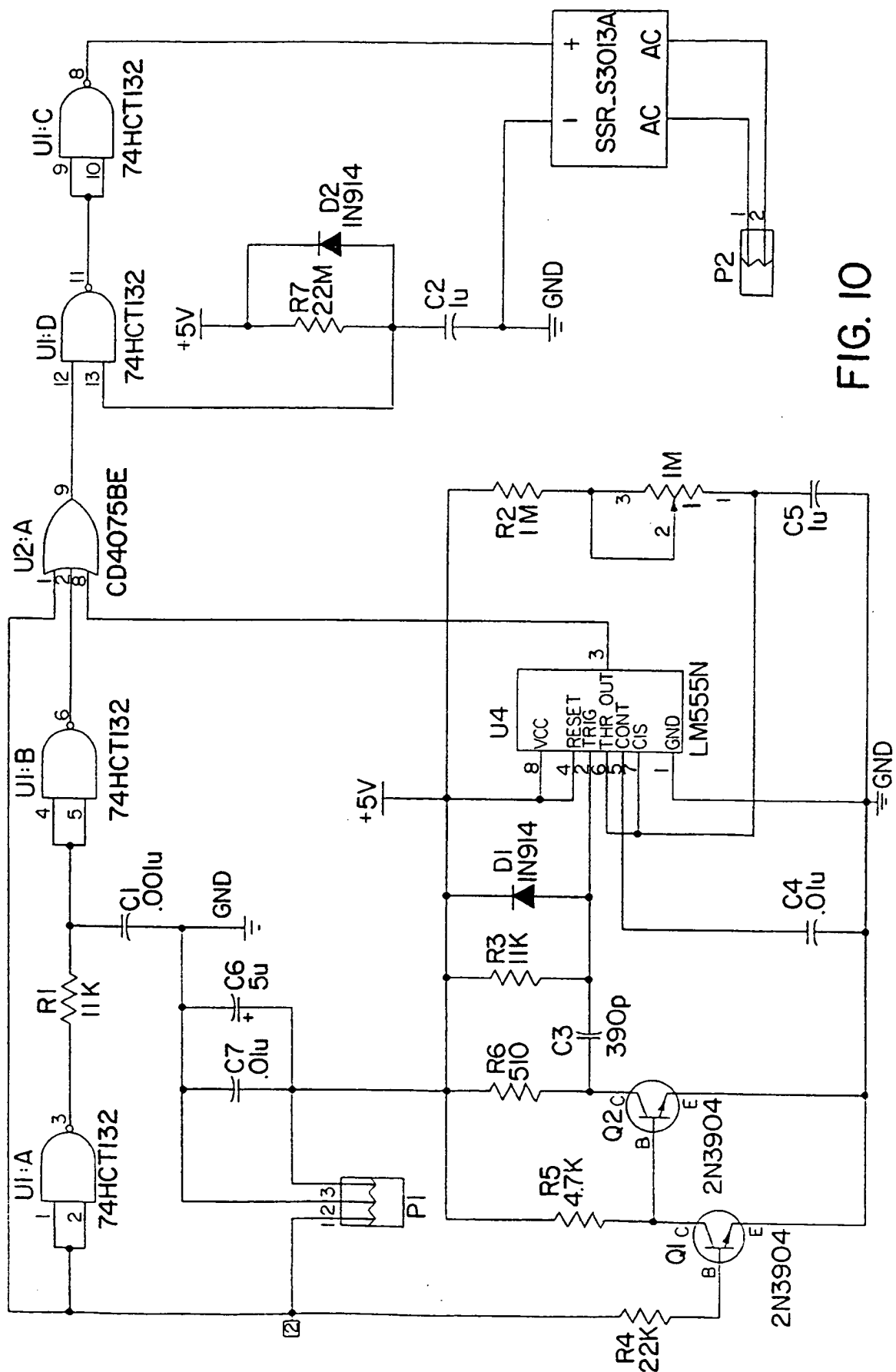


FIG. 10

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 96/10264

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 B01L3/00 C12M1/12 G01N35/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 B01L G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO,A,91 11245 (BIOCOM S A) 8 August 1991 see page 10, line 18 - page 11, line 15 see page 16, line 8 - page 17, line 22 ---	1
A	WO,A,92 02303 (PHARMACIA LKB BIOTECHNOLOGY AB) 20 February 1992 see page 7, line 20 - page 8, line 18; figures 1,3,4 --- -/--	1

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

Z document member of the same patent family

Date of the actual completion of the international search

20 September 1996

Date of mailing of the international search report

02.10.96

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl,
Fax (+ 31-70) 340-3016

Authorized officer

Hocquet, A

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 96/10264

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>US,A,5 380 437 (BERTONCINI JOSEPH) 10 January 1995 see column 17, line 16 - line 29; figure 15 see column 19, line 47 - line 63 see column 20, line 39 - line 46 see column 23, line 53 - column 24, line 2 see column 24, line 45 - line 54 see column 26, line 26 - column 27, line 45</p>	1
A	<p>--- US,A,4 879 431 (BERTONCINI JOSEPH) 7 November 1989 see column 10, line 39 - line 63; figures 1-3</p>	1
A	<p>--- WO,A,83 00819 (PROLIC AG ;BOEHME EIKE (AT); RUBENZER PETER (AT)) 17 March 1983 see page 6, line 18 - page 7, line 10</p>	1
A	<p>--- CHEMOMETRICS AND INTELLIGENT LABORATORY SYSTEMS, vol. 26, no. 2, 1 November 1994, pages 107-113, XP000476921 KOLB A J: "THE ROLE OF MICROPLATE SELECTION AND ASSAY DESIGN IN THE APPLICATION OF AUTOMATION AND ROBOTICS" see paragraph 6; figure 5</p>	1,13
A	<p>--- WO,A,91 16979 (SOURCE SCIENTIFIC SYSTEMS) 14 November 1991 see page 3, line 31 - page 4, line 7; figures 1,3,9</p>	13
A	<p>--- US,A,4 493 896 (LA MOTTE, III) 15 January 1985 see column 4, line 51 - column 5, line 5</p>	1
A	<p>--- GB,A,2 246 081 (BIO-RAD) 22 January 1992 see page 6, line 6 - line 19; figures 1,4</p> <p>-----</p>	1